Protocol of TRE-GH3 GH3 Cell-based Assay for High-throughput Screening

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| **DOCUMENT:** |  | TRE-GH3\_TOX21\_SLP\_Version1.0 |
| **TITLE:** |  | Protocol of TRE-GH3 GH3 Cell-based Assay for High-throughput Screening |

**ASSAY RFERENCES:**

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| --- | --- | --- | --- | --- | --- | --- |
| Assay Target | Cell Lines | Species | Tissue of Origin | Assay Readout | Assay Provider | Toxicity Pathway |
| Thyroid receptor: full  (Endogenous) | GH3 | Rat | Pituitary tumor GH3 | Luminescence | Dr. Murk | NR signaling |

**QUALITY CONTROL PRECAUTIONS:**

1. -

**MATERIALS and INSTRUMENTS:**

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| Supplies/Medium/Reagent | Manufacturer | Vender/Catalog Number |
| DMEM:F12 | -Invitrogen | -Gibco, 10565 |
| Fetal Bovine Serum | -Hyclone | -Hyclone, SH30071.03 |
| Penicillin/Streptomycin (antibiotic) | -Invitrogen | -Invitrogen, 15140 |
| insulin | Sigma | Sigma, I6634 |
| ethanolamine | Sigma | Sigma, E0135 |
| sodium selenite | -Sigma | -Sigma, S5261 |
| human apo-Transferrin | Sigma | -Sigma, T2036 |
| bovine serum albumin | -Sigma | Sigma, A9647 |
| -TrypLE Express | -Invitrogen | -Invitrogen, 12605 |
| -Phosphate-buffered saline without calcium and magnesium | -Invitrogen | -Invitrogen, 14190 |
| -Recovery Cell Culture freezing medium | -Invitrogen | Invitrogen, 12648 |
| -centrifuge | Sorvall legend XTR | -Thermo Fisher Science, 75004520 |
| -BioRAPTR, Microfluidic Workstation | -Beckmen | - |
| -Pintool | Kalypsys | - |
| -White, TC, sterile 1536-well assay plates | -Greiner Bio-One | Greiner, 789173-F |
| -Viewlux plate reader | PerkinElmer | - |
| -T3 (Agonist control compound) | Calbiochem | Calbiochem, 642511 |
| -DMSO | -AMRESCO | -KD Medical, RGE-3070 |
| -One-Glo | -Promega | -Promega, E6120 |

**PROCEDURE:**

1. Cell handling:

1.1. Media Required:

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Component | Growth Medium | Assay Medium | Thaw Medium | Freezing Medium |
| -Recovery Cell Culture Medium | - | - | - | -100% |
| -DMEM: F12 | -90% | -100% | -90% | - |
| -fetal bovine serum | -10% | - | -10% | - |
| -Penicillin-Streptomycin | -100U/mL-100ug/mL | - | --100U/mL-100ug/mL | - |
| -insulin | - | -10ug/mL | - | - |
| -ethanolamine | - | -10uM | - | - |
| -sodium selenite | - | -10ng/mL | - | - |
| - human apo-Transferrin | - | -10ug/mL | - | - |
| -bovine serum albumin | - | -500ug/mL | - | - |

1.2. Thawing method

1.2.1 Place 14 mL of pre-warmed thaw medium into a T75 flask.

1.2.2 Remove the vial of cells to be thawed from liquid nitrogen and thaw rapidly by placing at 37C in a water bath with gentle agitation for 1-2 minutes. Do not submerge vial in water.

1.2.3 Decontaminate the vial by wiping with 70% ethanol before opening in a biological safety cabinet.

1.2.4 Transfer the vial contents drop-wise into 10 mL of Thaw Medium in a sterile 15-mL conical tub

1.2.5 -Centrifuge cells at 1000 rpm for 4 mins

1.2.6 Transfer contents to the T75 tissue culture flask containing Thaw Medium and place flask in a humidified 37C/5% CO2 incubator.

1.2.7 Switch to growth medium at first passage.

1.3. Propagation method

1.3.1 Aspirate medium, rinse once in DPBS, add TrypLE Express (3 mL for a T75 flask and 5 mL for a T175 flask and 7.5 mL for T225 flask) and swirl to coat the cell evenly.

1.3.2 Add an equal volume of Growth Medium to inactivate Trypsin after 2-3 mins incubation at 37C.

1.3.3 Centrifuge cells at 1000 rpm for 4 mins and resuspend in Growth Medium.

1.3.4 Cell should be passage or fed at least twice a week.

2. Assay Protocol

2.1 Harvest cells from culture in Growth Medium and resuspend in assay medium

2.2 Dispense 1500 cells/5 µL/well into 1536-well tissue treated white solid plates using a BioRAPTR dispenser.

2.3 After the cells were incubated at 37C for 4 hrs, 23 nL of compounds dissolved in DMSO, positive controls or DMSO were transferred to the assay plate by a PinTool resulting in a 217-fold dilution.

2.4 Incubate the plates for 24 hrs at 37C.

2.5 Add 5 µL of One-Glo to each well using a BioRAPTR dispenser and incubate the plate at room temperature for 30 mins.

2.6 -Measure luminescence using Viewlux

3. Assay Performance

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| **GH3-TRE**  **(T3; Agonist control)** | **Online Validation**  **Agonist**  **(Mean ± SD)** |
| EC50 | 0.67 ± 0.20 nM  (n = 27) |
| S/B | 6.64 ± 0.28 |
| CV (%) ⃰ | 8.75 ± 1.80  (n = 18) |
| Z’ | 0.77 ± 0.04 |

⃰ CV values shown represent average of DMSO plates and low concentration plates only.